

FORM PTO-1390  
(REV. 11-2000)

U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER

TRANSMITTAL LETTER TO THE UNITED STATES  
DESIGNATED/ELECTED OFFICE (DO/EO/US)  
CONCERNING A FILING UNDER 35 U.S.C. 371

Mo-6546/LeA 33,254

U.S. APPLICATION NO. (If known, see 37 CFR 1.5

09/913812  
To be Assigned

INTERNATIONAL APPLICATION NO.

INTERNATIONAL FILING DATE

PRIORITY DATE CLAIMED

PCT/EP00/01028

09 February 2000 (9.02.00)

19 February 1999 (19.02.99)

TITLE OF INVENTION METHOD FOR ISOLATING NUCLEIC ACIDS

APPLICANT(S) FOR DO/EO/US 1) Wolfgang Podszun; 2) Rainer Neumann

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
  - a. ☒ is attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ has been communicated by the International Bureau.
  - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
  - a. ☒ is attached hereto.
  - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
  - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
  - b. ☐ have been communicated by the International Bureau.
  - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
  - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

## Items 11 to 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A **FIRST** preliminary amendment.
14. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. ☒ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information:

PTO-1449 and references listed therein; Preliminary Amendment w/Abstract

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)  
To be Assigned

09/913812

INTERNATIONAL APPLICATION NO  
PCT/EP00/01028ATTORNEY'S DOCKET NUMBER  
Mo-6546/LeA 33,25421. ☒ The following fees are submitted:**BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):**Neither international preliminary examination fee (37 CFR 1.482)  
nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO  
and International Search Report not prepared by the EPO or JPO ..... \$1000.00International preliminary examination fee (37 CFR 1.482) not paid to  
USPTO but International Search Report prepared by the EPO or JPO ..... \$860.00International preliminary examination fee (37 CFR 1.482) not paid to USPTO  
but international search fee (37 CFR 1.445(a)(2)) paid to USPTO ..... \$710.00International preliminary examination fee (37 CFR 1.482) paid to USPTO  
but all claims did not satisfy provisions of PCT Article 33(1)-(4) ..... \$690.00International preliminary examination fee (37 CFR 1.482) paid to USPTO  
and all claims satisfied provisions of PCT Article 33(1)-(4) ..... \$100.00**ENTER APPROPRIATE BASIC FEE AMOUNT =****CALCULATIONS PTO USE ONLY**

\$ 860.00

Surcharge of \$130.00 for furnishing the oath or declaration later than ☐ 20 ☐ 30  
months from the earliest claimed priority date (37 CFR 1.492(e)).

\$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$
Total claims	12 -20 =	0	x \$18.00	\$ 0.00
Independent claims	7 -3 =	4	x \$80.00	\$ 320.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00	\$ 0.00
<b>TOTAL OF ABOVE CALCULATIONS =</b>				\$ 1,180.00
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	\$ 0.00
<b>SUBTOTAL =</b>				\$ 1,180.00
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$
<b>TOTAL NATIONAL FEE =</b>				\$ 1,180.00
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$ 0.00
<b>TOTAL FEES ENCLOSED =</b>				\$ 1,180.00
			<b>Amount to be refunded:</b>	\$
			<b>charged:</b>	\$

\$

a. ☐ A check in the amount of \$ \_\_\_\_\_ to cover the above fees is enclosed.b. ☒ Please charge my Deposit Account No. 13-3848 in the amount of \$ 1,180.00 to cover the above fees.  
A duplicate copy of this sheet is enclosed.c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3848. A duplicate copy of this sheet is enclosed.d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.**NOTE:** Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO



00157

PATENT TRADEMARK OFFICE

Richard E.L. Henderson  
SIGNATURERichard E.L. Henderson  
NAME31,619  
REGISTRATION NUMBER

09/913812  
518 Rec'd PCT/PTO 17 AUG 2001

PATENT APPLICATION  
Mo-6546  
LeA 33,254

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF )  
 )  
WOLFGANG PODSZUN ET AL )  
 )  
SERIAL NUMBER: TO BE ASSIGNED )  
 )  
FILED: HEREWITH )  
 )  
TITLE: METHOD FOR ISOLATING )  
NUCLEIC ACIDS )

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D.C. 20231

Sir:

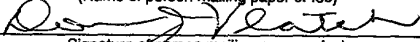
Prior to examination, please amend the application as follows.

"Express Mail" mailing label number ET146885765US  
Date of Deposit August 17, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231

Donna J. Veatch

(Name of person mailing paper or fee)



(Signature of person mailing paper or fee)

09/913812

IN THE CLAIMS:

Please replace the heading at page 19, line 1, with --WHAT IS CLAIMED IS:--

Please cancel Claims 1-11 and add Claims 12-23.

--12. A method for isolating nucleic acids from a sample containing nucleic acids comprising

- (A) mixing, at a pH of 7 or less, the sample with a water-insoluble polymer that is not ionic in the basic and neutral range, is a bead polymer having an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 0.3 to 30% by weight of crosslinker, and
  - (c) 0 to 93% by weight of vinyl monomer,thereby absorbing the nucleic acids,
- (B) separating the water-insoluble polymer on which is absorbed the nucleic acids, and
- (C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, thereby liberating the adsorbed nucleic acids.

13. A method according to Claim 12 wherein the sample is a biological material that is lysed after step (A).

14. A method according to Claim 12 wherein the polymer is
- (1) a water-insoluble, macroporous bead polymer that has an average particle size of from 3 to 100  $\mu\text{m}$  and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consists of polymerized units of
    - (a) 5 to 98% by weight of amino monomer,
    - (b) 0.3 to 30% by weight of crosslinker, and
    - (c1) 0 to 93% by weight of hydrophobic vinyl monomer, or
  - (2) a bead polymer that is able to swell in water well, has an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of

- (a) 5 to 79.5% by weight of amino monomer,
- (b) 0.3 to 10% by weight of crosslinker, and
- (c2) 10 to 93% by weight of hydrophilic vinyl monomer.

15. A method according to Claim 12 wherein the polymer is

- (1) a water-insoluble, macroporous bead polymer that has an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm, and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consists of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 2 to 30% by weight of crosslinker, and
  - (c1) 0 to 93% by weight of hydrophobic vinyl monomer, or
- (2) a bead polymer that is insoluble in water but swellable in water, has an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of
  - (a) 5 to 79.5% by weight of amino monomer,
  - (b) 0.3 to 10% by weight of crosslinker, and
  - (c2) 10 to 93% by weight of hydrophilic vinyl monomer.

16. A water-insoluble, macroporous bead polymer that has an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm, and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consists of polymerized units of

- (a) 5 to 98% by weight of amino monomer,
- (b) 2 to 30% by weight of crosslinker, and
- (c1) 0 to 93% by weight of hydrophobic vinyl monomer.

17. A bead polymer that is insoluble in water but swellable in water, has an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of

- (a) 5 to 79.5% by weight of amino monomer,
- (b) 0.3 to 10% by weight of crosslinker, and
- (c2) 10 to 93% by weight of hydrophilic vinyl monomer.

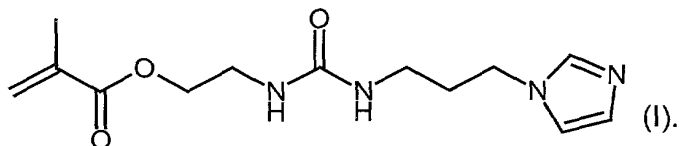
18. A method for preparing water-insoluble, macroporous bead polymers that have an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm, and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  comprising

- (1) dispersing, in an aqueous medium using a protective colloid, a mixture of
  - (a) 5 to 98 parts by weight of amino monomer,
  - (b) 2 to 30 parts by weight of crosslinker,
  - (c1) 0 to 93 parts by weight of hydrophobic vinyl monomer,
  - (d) 10 to 150 parts by weight of porogen, and
  - (e) 0.1 to 2.5 parts by weight of free-radical former,
- (2) polymerizing the resulting dispersion by heating to the decomposition temperature of the free-radical former, and
- (3) thereafter removing the porogen by extraction and/or evaporation.

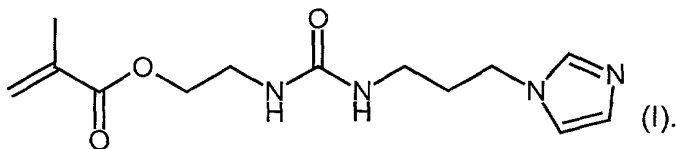
19. A method for preparing bead polymers that are insoluble but swellable in water and have an average particle size of from 3 to 100  $\mu\text{m}$  comprising

- (1) dispersing, in an aqueous medium using a protective colloid, a mixture of
  - (a) 5 to 79.7% by weight of amino monomer,
  - (b) 0.3 to 10% by weight of crosslinker,
  - (c2) 10 to 93% by weight of hydrophilic vinyl monomer,
  - (d) 10 to 150 parts by weight of solvent, and
  - (e) 0.1 to 2.5 parts by weight of free-radical former
- (2) polymerizing the resulting dispersion by heating to the decomposition temperature of the free-radical former, and
- (3) thereafter removing the porogen by extraction and/or evaporation.

20. A method according to Claim 12 wherein the amino monomer is a compound of formula (I)



21. An amino monomer of formula (I)



22. A method for preparing the amino monomer of Claim 21 comprising reacting 2-isocyanatoethyl methacrylate with 3-aminopropylimidazole.

23. A composition for isolating nucleic acids from a sample from a sample containing nucleic acids comprising

- (1) water-insoluble macroporous bead polymers that have an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm, and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consist of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 2 to 30% by weight of crosslinker, and
  - (c1) 0 to 93% by weight of hydrophobic vinyl monomer, or
- (2) bead polymers that are insoluble but swellable in water, have an average particle size of from 3 to 100  $\mu\text{m}$ , and consist of polymerized units of
  - (a) 5 to 79.5% by weight of amino monomer,
  - (b) 0.3 to 10% by weight of crosslinker, and
  - (c2) 10 to 93% by weight of hydrophilic vinyl monomer.--

IN THE ABSTRACT:

Please add an Abstract as new page 24 to read as follows:

--WO 00/49031

PCT/EP00/01028

- 24 -

METHOD FOR ISOLATING NUCLEIC ACIDS

ABSTRACT OF THE DISCLOSURE

The invention relates to a method for isolating nucleic acids from a sample containing nucleic acids by

- 0913812-0404  
FOI# 288765
- (A) mixing, at a pH of 7 or less, the sample with a water-insoluble polymer that is not ionic in the basic and neutral range, is a bead polymer having an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of
    - (a) 5 to 98% by weight of amino monomer,
    - (b) 0.3 to 30% by weight of crosslinker, and
    - (c) 0 to 93% by weight of vinyl monomer,thereby absorbing the nucleic acids,
  - (B) separating the water-insoluble polymer on which is absorbed the nucleic acids, and
  - (C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, thereby liberating the adsorbed nucleic acids.



REMARKS

Applicants hereby offer preliminary amendments to the present application to place the application in better form for allowance.

Applicants have canceled Claims 1-11 in favor of replacement Claims 12-23 to correct certain informalities (including the replacement of use Claim 10 with new method Claim 15), to clarify the intended meaning of the claims without altering their scope, and to add a method claim (i.e., new Claim 20) that specifies the particular amino monomer claimed in Claim 21. Applicants respectfully submit that the claims remain fully supported in the specification.

Applicants have added an Abstract that summarizes the subject matter of their invention. A copy of the new Abstract is separately attached.

In view of the preceding amendments and remarks, allowance of the claims is respectfully requested.

Respectfully submitted,

By Richard E.L. Henderson  
Richard E.L. Henderson  
Attorney for Applicants  
Reg. No. 31,619

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s:/sr/relh0349

## ANNOTATED VERSION OF AMENDMENTS

### IN THE CLAIMS:

The heading for the claims at page 19 , line 1, has been changed from

**"Patent claims"** to **--WHAT IS CLAIMED IS:--**

Claims 1-11 have been canceled in favor of replacement Claims 12-23.

--12. A method for isolating nucleic acids from a sample containing nucleic acids comprising

- (A) mixing, at a pH of 7 or less, the sample with a water-insoluble polymer that is not ionic in the basic and neutral range, is a bead polymer having an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 0.3 to 30% by weight of crosslinker, and
  - (c) 0 to 93% by weight of vinyl monomer,thereby absorbing the nucleic acids,
- (B) separating the water-insoluble polymer on which is absorbed the nucleic acids, and
- (C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, thereby liberating the adsorbed nucleic acids.

13. A method according to Claim 12 wherein the sample is a biological material that is lysed after step (A).

14. A method according to Claim 12 wherein the polymer is

- (1) a water-insoluble, macroporous bead polymer that has an average particle size of from 3 to 100  $\mu\text{m}$  and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consists of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 0.3 to 30% by weight of crosslinker, and
  - (c1) 0 to 93% by weight of hydrophobic vinyl monomer, or
- (2) a bead polymer that is able to swell in water well, has an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of



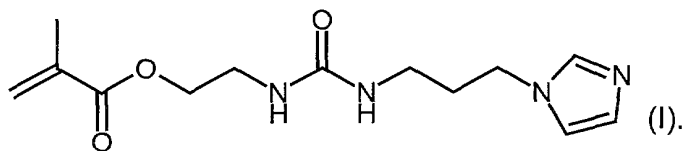
18. A method for preparing water-insoluble, macroporous bead polymers that have an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm, and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  comprising

- (1) dispersing, in an aqueous medium using a protective colloid, a mixture of
  - (a) 5 to 98 parts by weight of amino monomer,
  - (b) 2 to 30 parts by weight of crosslinker,
  - (c1) 0 to 93 parts by weight of hydrophobic vinyl monomer,
  - (d) 10 to 150 parts by weight of porogen, and
  - (e) 0.1 to 2.5 parts by weight of free-radical former,
- (2) polymerizing the resulting dispersion by heating to the decomposition temperature of the free-radical former, and
- (3) thereafter removing the porogen by extraction and/or evaporation.

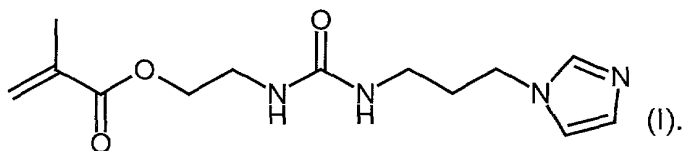
19. A method for preparing bead polymers that are insoluble but swellable in water and have an average particle size of from 3 to 100  $\mu\text{m}$  comprising

- (1) dispersing, in an aqueous medium using a protective colloid, a mixture of
  - (a) 5 to 79.7% by weight of amino monomer,
  - (b) 0.3 to 10% by weight of crosslinker,
  - (c2) 10 to 93% by weight of hydrophilic vinyl monomer,
  - (d) 10 to 150 parts by weight of solvent, and
  - (e) 0.1 to 2.5 parts by weight of free-radical former
- (2) polymerizing the resulting dispersion by heating to the decomposition temperature of the free-radical former, and
- (3) thereafter removing the porogen by extraction and/or evaporation.

20. A method according to Claim 12 wherein the amino monomer is a compound of formula (I)



21. An amino monomer of formula (I)



22. A method for preparing the amino monomer of Claim 21 comprising reacting 2-isocyanatoethyl methacrylate with 3-aminopropylimidazole.

23. A composition for isolating nucleic acids from a sample from a sample containing nucleic acids comprising

- (1) water-insoluble macroporous bead polymers that have an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm, and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consist of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 2 to 30% by weight of crosslinker, and
  - (c1) 0 to 93% by weight of hydrophobic vinyl monomer, or
- (2) bead polymers that are insoluble but swellable in water, have an average particle size of from 3 to 100  $\mu\text{m}$ , and consist of polymerized units of
  - (a) 5 to 79.5% by weight of amino monomer,
  - (b) 0.3 to 10% by weight of crosslinker, and
  - (c2) 10 to 93% by weight of hydrophilic vinyl monomer.--

#### **IN THE ABSTRACT:**

An Abstract has been added as new page 24 as follows:

--WO 00/49031

PCT/EP00/01028

- 24 -

#### **METHOD FOR ISOLATING NUCLEIC ACIDS**

#### **ABSTRACT OF THE DISCLOSURE**

The invention relates to a method for isolating nucleic acids from a sample containing nucleic acids by

- (A) mixing, at a pH of 7 or less, the sample with a water-insoluble polymer that is not ionic in the basic and neutral range, is a bead polymer having an average particle size of from 3 to 100  $\mu\text{m}$ , and consists of polymerized units of
  - (a) 5 to 98% by weight of amino monomer,
  - (b) 0.3 to 30% by weight of crosslinker, and
  - (c) 0 to 93% by weight of vinyl monomer,thereby absorbing the nucleic acids,
- (B) separating the water-insoluble polymer on which is absorbed the nucleic acids, and
- (C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, thereby liberating the adsorbed nucleic acids.--

513 Rec'd PCT/PTO 17 AUG 2001

- 1 -

Method for isolating nucleic acids

5 The invention relates to a method for separating off and selectively liberating nucleic acids by use of special bead polymers

So-called genetic diagnosis has become increasingly important recently.

Genetic diagnosis has become involved in the diagnosis of human diseases (inter alia detection of pathogens, detection of genome mutations, analysis of circulating tumor cells and identification of risk factors for predisposition to a disease). However, genetic diagnosis is also now finding applications in veterinary medicine, environmental analysis and food testing. A further area of application comprises investigations in institutes of pathology/cytology or within the framework of forensic problems. However, genetic diagnosis is now employed also for the purposes of quality and process control (for example investigations of blood samples for freedom from pathogens), and legislation is planned to extend the number of tests already required now by law in the future.

Methods also employed in genetic diagnosis (such as, for example, hybridization and amplification techniques such as PCR, bDNA or NASBA, TMA technology) are also among the routine methods in fundamental scientific studies.

The increasingly wide use of nonradioactive detection methods, which also play a part in genetic diagnosis, leads to the expectation that genetic diagnosis will be used even more widely in future than at present.

An important step in genetic diagnosis is the obtaining of gene samples from biological materials such as cells, blood, sputum, CSF, serum or urine.

"Express Mail" mailing label number ET146885765US  
Date of Deposit August 17, 2001

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner of Patents and Trademarks, Washington, D.C. 20231

Donna J. Veatch

(Name of person mailing paper or fee)

Signature of person mailing paper or fee

- 2 -

The binding of nucleic acids to polyquaternary cationic polymers is disclosed in US-A-4 046 750. However, the binding is irreversible, so that the adsorbed nucleic acids cannot be liberated again in this method.

5 US-A-4 055 469 discloses a method for purifying enzymes where nucleic acids and unwanted proteins are precipitated with the aid of water-soluble cationic polymers.

US-A-4 839 231 discloses supports which are coated with vinylpyridine polymer and are able to adsorb proteins and nucleic acids. However, the capacity of the supports is rather low and the adsorption of the nucleic acids is not quantitative.

10 WO-A-91/05606 describes a silanized, porous support material with hydroxyalkylamino groups for chromatographic separation of nucleic acids. However, this material is less well suited to rapid and maximally quantitative separation of nucleic acids out of biological materials.

15 DE-A-4 139 664 describes an apparatus and a method for isolating and purifying nucleic acids with the aid of anion exchangers. The disadvantages of this method are that it is possible to desorb the nucleic acids off the anion exchanger only with buffer solutions of high ionic strength, and the separation of the salts out of the buffer solution requires additional preparation steps.

20 DE-A-4 333 805 claims the extraction of nucleic acids from a sample with the aid of water-soluble carriers such as dextran, acrylamide or carboxymethylcellulose and other reagents, with the nucleic acids being precipitated.

25 EP-A-0 707 077 (corresponds to US-A-5 582 988) describes a method for isolating nucleic acids from biological material using soluble, weakly basic polymer. In this method, a precipitate is generated from a soluble, weakly basic polymer and the nucleic acid in an acidic pH range, the precipitate is separated from the

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unprecipitated constituents of the biological material, and washed, and the nucleic acid is liberated again from the precipitate by adjusting a basic pH.

One disadvantage of the methods of DE-A-4 333 805 and EP-A-0 707 077 is that the manipulation, in particular the separation and purification of the precipitate, is difficult and very time consuming. These methods can moreover be carried out using automatic analyzers only under difficult conditions or not at all.

WO-A-96/18731 describes a method for isolating nucleic acids with the aid of a detergent and of a solid support. Since solid supports without pores and without swellability are employed, the binding capacity of the supports is relatively low.

WO-A-97/08547 describes a method for isolating nucleic acids in which the nucleic acids are bound to a solid hydrophilic organic polymer without effective positive charge, for example to a cellulose. In this method, the binding is effected by weak forces such as Van der Waals interactions.

WO-A-97/34909 describes a method for isolating nucleic acids by use of a specific particulate polymer with a lower critical solubility temperature (LCST) of 25-45° C. A disadvantage of this method is that buffers of high ionic strength must be used to liberate the nucleic acids but may interfere with further use of the nucleic acids. Because of the small size of the particles used, from 0.05 to 2 µm, moreover, the processing is time-consuming and difficult to automate.

Despite the numerous previously described methods for isolating nucleic acids, there is still a pressing need for a simple method, which can be automated if possible, for the effective separation of nucleic acids from biological material, and the liberation again thereof.

It has now been found, surprisingly, that certain water-insoluble bead polymers of polymerized units of amino monomer, crosslinker and vinyl monomer are outstandingly suitable for isolating nucleic acids.

5 The invention relates to a method for isolating nucleic acids from a sample, comprising the following steps

10 A) mixing the sample with a water-insoluble polymer which is not ionic in the basic and neutral range, at a pH of 7 or less, with the nucleic acids being adsorbed,

B) separating off the water-insoluble polymer and

15 C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, with the adsorbed nucleic acids being liberated,

characterized in that the water-insoluble polymer is a bead polymer with an average particle size of from 3 to 100  $\mu\text{m}$  and consists of polymerized units of

- 20 a) 5 to 98% by weight of amino monomer  
b) 0.3 to 30% by weight of crosslinker and  
c) 0 to 93% by weight of vinyl monomer.

25 Where appropriate, in a step interpolated after method step A) the biological material is lysed.

The present invention preferably relates to a method for isolating nucleic acids from a sample, comprising the steps

A) mixing the sample with a water-insoluble polymer which is not ionic in the basic and neutral range, at a pH of 7 or less, with the nucleic acids being adsorbed,

5 B) separating off the water-insoluble polymer and

C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, with the adsorbed nucleic acids being liberated,

10

characterized in that the water-insoluble polymer is a bead polymer with an average particle size of from 3 to 100  $\mu\text{m}$  and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consists of polymerized units of

15

- a) 5 to 98% by weight of amino monomer
- b) 0.3 to 30% by weight of crosslinker and
- c1) 0 to 93% by weight of hydrophobic vinyl monomer

20

or in that the water-insoluble polymer consists of

bead polymer which is able to swell in water well and has an average particle size of from 3 to 100  $\mu\text{m}$ , and which consists of polymerized units of

25

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer.

30

The present invention particularly preferably relates to a method for isolating nucleic acids from a sample, comprising steps A), B) and C) defined above, characterized in that the water-insoluble polymer is a macroporous bead polymer with a particle size of from 3 to 100  $\mu\text{m}$  and a pore diameter in the range from 10 to 1 000 nm, which

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has a specific surface area determined by the BET method of 5 to 500 m<sup>2</sup>/g (very particularly preferably 20 to 200 m<sup>2</sup>/g) and consists of polymerized units of

- a) 5 to 98% by weight of amino monomer
- b) 2 to 30% by weight of crosslinker and
- c1) 0 to 93% by weight of hydrophobic vinyl monomer

or in that the water-insoluble polymer consists of

bead polymer which is able to swell in water well and has an average particle size of from 3 to 100 µm, and which consists of polymerized units of

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer.

The method of the invention is suitable for the isolation and/or purification of nucleic acids of varying origin, for example from cells, tissue materials, blood or pathogens. Before the isolation of the nucleic acids, the material to be investigated is disrupted by techniques known per se, such as, for example, disruption by protease digestion, resulting in a sample, a lysate, suitable for subsequent steps A to C. Where appropriate, the biological material is lysed in a step interpolated after method step A). Further suitable disruption methods have been described in DE-A-4 333 805.

The sample is mixed with a water-insoluble polymer at a pH of 7 or less, preferably in the range 2 – 6, particularly preferably in the range 2 – 3, at room temperature. The water-insoluble polymer is separated off for example by filtration or by centrifugation. The complex of nucleic acid and polymer which is obtained in this way can then be purified by washing with suitable buffers such as, for example, TE.

To liberate the bound nucleic acids from the complex, the pH of the complex is then adjusted to pH values above 7, preferably of 8 – 14, particularly preferably in the range 12 – 14.

5 The bead polymers of the invention provide higher rates of adsorption and liberation again than the soluble polymers disclosed in EP-A-0 707 077. The isolation can be carried out more easily, i.e. with fewer working steps and in shorter times. The purity of the isolated nucleic acids is higher and, in particular, they contain fewer inhibiting byproducts so that amplification of the nucleic acids, for example by the so-called  
10 "PCR reaction" and the "RT-PCR", takes place particularly well. The method of the invention is also superior to the method described in EP-A-0 707 077 in relation to restriction enzyme digestion of the nucleic acids obtained.

The present invention further relates to the macroporous bead polymers,  
15 characterized in that they have an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consist of polymerized units of

- 20 a) 5 to 98% by weight of amino monomer  
b) 2 to 30% by weight of crosslinker and  
c1) 0 to 93% by weight of hydrophobic vinyl monomer,

and

25 the bead polymers which are insoluble but swellable in water, characterized in that they have an average particle size of from 3 to 100  $\mu\text{m}$  and consist of polymerized units of

- 30 a) 5 to 79.7% by weight of amino monomer  
b) 0.3 to 10% by weight of crosslinker and  
c1) 10 to 93% by weight of hydrophilic vinyl monomer.

The present invention further relates to a method for preparing water-insoluble, macroporous bead polymers with an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$ , characterized in that a mixture of

- a) 5 to 98 parts by weight of amino monomer
- b) 2 to 30 parts by weight of crosslinker
- c1) 0 to 93 parts by weight of hydrophobic vinyl monomer
- d) 10 - 150 parts by weight of porogen and
- e) 0.1 - 2.5 parts by weight of free-radical former

is dispersed in an aqueous medium using a protective colloid, subsequently the resulting dispersion is polymerized by heating to the decomposition temperature of the free-radical former and, after the polymerization has taken place, the porogen is removed by extraction and/or evaporation,

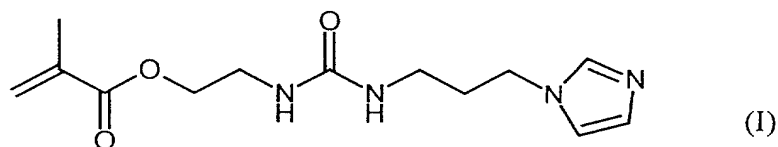
and a method for preparing bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100  $\mu\text{m}$ , characterized in that a mixture of

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer
- d) 10-150 parts by weight of solvent and
- e) 0.1 - 2.5 parts by weight of free-radical former

is dispersed in an aqueous medium using a protective colloid, subsequently the resulting dispersion is polymerized by heating to the decomposition temperature of the free-radical former and, after polymerization has taken place, the solvent is removed by extraction and/or evaporation.

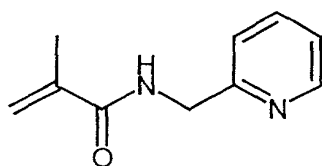
Amino monomers (a) for the purposes of the invention are polymerizable, ethylenically unsaturated compounds with at least one primary, secondary or tertiary amino group. The secondary or tertiary amino group may moreover be part of a cycloaliphatic or aromatic ring. Examples which may be mentioned are N-vinylimidazole, N-vinylbenzimidazole, 2-vinylpyridine and 4-vinylpyridine. Very suitable amino monomers are also the derivatives of acrylic acid and methacrylic acid, such as, for example, 2-aminoethyl methacrylate, N,N-dimethylaminoethyl methacrylate, N,N-dimethylaminopropyl methacrylate, N,N-dimethylaminoethyl acrylate, N-tert-butylaminopropyl methacrylate, N-(3-aminopropyl)methacrylamide, N-(3-imidazolylpropyl)methacrylamide, N-(2-imidazolethyl)methacrylamide, N-(3-aminopropyl)acrylamide, N-(3-imidazolylpropyl)acrylamide, N-(2-imidazolethyl)acrylamide, N-(1,1-dimethyl-3-imidazolylpropyl)methacrylamide, N-(1,1-Dimethyl-3-imidazolylpropyl)acrylamide, N-(3-benzimidazolylpropyl)methacrylamide and (3-benzimidazolylpropyl)acrylamide.

Very suitable amino monomers are also the products of the reaction of isocyanatoethyl (meth)acrylate and imidazolylalkylamines, such as, for example, the amino monomer of formula (I) which is novel within the framework of the present invention

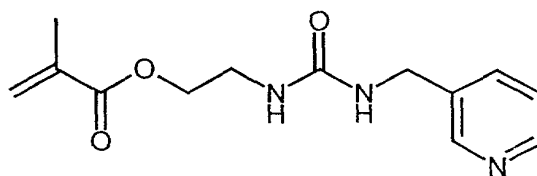


Further suitable amino monomers according to the present invention are the pyridine derivatives of the formulae (II) and (III)

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(II)



(III).

Derivatives of styrene and  $\alpha$ -methylstyrene with amino groups are also very suitable.

5 Examples which may be mentioned are: 4-N,N-dimethylaminostyrene, 2-N,N-dimethylaminostyrene, 4-N,N-diethylaminostyrene and 4-N,N-bis(2-hydroethyl)aminostyrene.

10 Crosslinkers (b) suitable according to the present invention are: ethylene glycol dimethacrylate, butanediol dimethacrylate, hexanediol dimethacrylate, pentaerythritol dimethacrylate, glycerol 1,2-dimethacrylate, glycerol 1,3-dimethacrylate, triethylene glycol dimethacrylate, tetraethylene glycol dimethacrylate, trimethylolpropane trimethacrylate, pentaerythritol trimethacrylate, pentaerythritol tetramethacrylate, ethylene glycol diacrylate, butanediol diacrylate, 15 pentaerythritol diacrylate, glycerol 1,3-diacrylate, triethylene glycol diacrylate, trimethylolpropane triacrylate, pentaerythritol triacrylate, pentaerythritol tetraacrylate, allyl methacrylate, allyl acrylate, methylene-N,N'-bisacrylamide, p-divinylbenzene and m-divinylbenzene.

20 Hydrophobic vinyl monomers (c1) which may be present in the bead polymer of the invention and are suitable for the purposes of the present invention are C<sub>1</sub>-C<sub>8</sub>-alkyl acrylates, C<sub>1</sub>-C<sub>8</sub>-alkyl methacrylates, such as, for example, methyl methacrylate or butyl acrylate, acrylonitrile, methacrylonitrile, vinyl chloride, vinylidene chloride, vinyl acetate and aromatic vinyl monomers such as, for example, styrene, 25 vinylnaphthalene, vinyltoluene, ethylstyrene,  $\alpha$ -methylstyrene, chlorostyrenes and vinylbenzyl chloride.



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Hydrophilic vinyl monomers (c2) suitable for the purposes of the present invention are, for example: 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 2-hydroxyethyl acrylate, 2-hydroxypropyl acrylate, triethylene glycol monomethacrylate, tetraethylene glycol monomethacrylate, acrylamide, methacrylamide and N,N-dimethylacrylamide.

Porogens are employed for the method of the invention for preparing water-insoluble macroporous bead polymers. Suitable for this purpose are liquid water-immiscible compounds which dissolve the monomers employed and precipitate the polymer formed. Examples which may be mentioned are aliphatic hydrocarbons such as hexane, heptane, octane, isooctane isododecane and alcohols such as octanol. The porogen is employed in amounts of from 10 to 150% by weight, preferably from 20 to 100% by weight, based on the total of the monomers and crosslinker employed.

Suitable and preferred free-radical formers for the purposes of the present invention are oil-soluble initiators. Examples which may be mentioned are: peroxy compounds such as dibenzoyl peroxide, dilauryl peroxide, bis (p-chlorobenzoyl peroxide), dicyclohexyl peroxydicarbonate, tert-butyl peroctoate, 2,5-bis(2-ethylhexanoylperoxy)-2,5-dimethylhexane and tert-amylperoxy-2-ethylhexane, in addition azo compounds such as 2,2'-azobis(isobutyronitrile), 2,2'-azobis(2,4-dimethylvalereonitrile) and 2,2'-azobis(2-methylisobutyronitrile). The initiators are generally used in amounts of from 0.05 to 2.5% by weight, preferably 0.2 to 1.5% by weight, based on the monomer mixture.

In the preparation of the macroporous gel bead polymers of the invention there is use, if appropriate, of protective colloids in the aqueous phase. Suitable protective colloids according to the present invention are natural and synthetic water-soluble polymers such as, for example, gelatin, starch, cellulose derivatives, in particular cellulose esters and cellulose ethers, polyvinyl alcohol, polyvinylpyrrolidone, polyacrylic acid, polymethacrylic acid and copolymers of acrylic acid, methacrylic acid, methacrylic esters and/or acrylic esters. Copolymers of methacrylic acid and

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methacrylic ester neutralized with alkali metal hydroxide are particularly suitable. The amount of protective colloid employed is generally 0.05 to 2% based on the aqueous phase, preferably 0.1 to 1%.

5 The aqueous phase may additionally contain where appropriate a buffer system. Preferred buffer systems adjust the pH of the aqueous phase at the start of the polymerization to a value between 12 and 5, preferably between 10 and 6. Under these conditions, dispersants with carboxyl groups are wholly or partly in the form of salts. The effect of the protective colloids is beneficially influenced in this way.  
10 Particularly suitable buffer systems contain phosphate or borate salts.

The amount of the aqueous phase is generally 75 to 1200% by weight, preferably 100 to 500% by weight, based on the total of monomers, crosslinker and porogen.

15 The stirring speed during the polymerization is important for adjusting the particle size. In the preparation method of the invention the size of the resulting bead polymers decreases with increasing stirrer speed. The exact stirrer speed for adjusting a particular predetermined bead size depends in the individual case greatly on the size of the reactor, the reactor geometry and the stirrer geometry. It has proved to be expedient to determine the necessary stirring speed by experiment. For laboratory  
20 reactors with a reaction volume of 3 liters and equipped with paddle stirrers, on use of copolymers of acrylic acid, methacrylic acid, acrylic esters and/or methacrylic esters as dispersant in general bead sizes of from 6 to 30  $\mu\text{m}$  are reached with speeds of from 300 to 500 rpm.

25 The polymerization temperature in the preparation method of the invention depends on the decomposition temperature of the initiator employed. It is generally between 50 and 150°C, preferably between 55 and 100°C. Polymerization takes 0.5 to some hours. It has proved appropriate to use a temperature program in which the  
30 polymerization is started at low temperature, for example 70°C, and the reaction temperature is raised as the polymerization reaction progresses.

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After the polymerization it is possible for the polymer to be isolated by conventional methods, for example by filtration or decantation, where appropriate after one or more washes, to be dried. The porogen can be removed during the drying. In the case of low-boiling porogens such as, for example, hexane, it is also possible to remove the porogen from the aqueous reaction mixture wholly or partly by distillation before isolating the bead polymer.

Preparation of the bead polymers capable of swelling in water takes place in analogy to the preparation of the macroporous bead polymers, employing hydrophilic vinyl monomers (c2) in place of the hydrophobic vinyl monomers (c1), and a solvent in place of the porogen.

Suitable solids are those which are immiscible with water and dissolve the monomers and the crosslinker and do not precipitate, but dissolve or swell, the polymer formed. Suitable solvents are toluene, xylene, tetrachloromethane, chloroform, methylene chloride, dichloroethane and ethyl acetate. The amount of auxiliary solvent is generally 10 to 200% by weight, preferably 10 to 150% by weight, particularly preferably 20 to 100% by weight, based on the total of monomers and crosslinker. If required, the auxiliary solvent can be removed for example by distillation after the polymerization. Toluene can be removed particularly simply by azeotropic distillation.

The bead polymers capable of swelling in water according to the invention have swelling indices of from 1.2 to 12, preferably 1.5 to 8, measured at 25°C and pH 7. The swelling index is defined as the quotient of the volume of the bead polymer swollen to saturation in water and the volume of the anhydrous bead polymer.

The present application also relates to compositions for isolating nucleic acids comprising water-insoluble macroporous bead polymers with an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm and a specific surface

area measured by the BET method of from 5 to 500 m<sup>2</sup>/g, consisting of polymerized units of

- 5 a) 5 to 98% by weight of amino monomer  
 b) 2 to 30% by weight of crosslinker  
 c1) 0 to 93% by weight of hydrophobic vinyl monomer

or of bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100 µm, consisting of polymerized units of

10

- a) 5 to 79.7% by weight of amino monomer  
 b) 0.3 to 10% by weight of crosslinker and  
 c2) 10 to 93% by weight of hydrophilic vinyl monomer.

15

These compositions are preferably in the form of aqueous dispersions with a solids content of 0.1 – 50%, particularly preferably 1 – 10%. The aqueous phase may, where appropriate, contain buffers which are preferably effective in the range 2 – 6.

20

Possible areas of use of a composition which is formulated, for example, as a test kit are the application examples already mentioned above, for example in the isolation of nucleic acids from cells, tissue materials, blood or pathogens, with all diagnostic questions in particular being involved. The described polymers are also understood as test kit for routine nucleic acid tests in microtiter plates and/or test tubes, or other formats such as, for example, within the framework of chip technology.

## Examples

### Example 1

#### 5     Preparation of the amino monomer of the formula 1

62.08 g (0.4 mol) of 2-isocyanatoethyl methacrylate were added dropwise over the course of 60 min to a stirred solution of 50.07 g (0.4 mol) of 3-aminopropylimidazole, 140 mg of 2,6-di-tert.butyl-4-methylphenol (stabilizer),  
10     140 mg of dibutyltin dilaurate (catalyst) in 250 ml of chloroform at 20°C with cooling. The mixture was then heated at 50°C until (about 5 h) no NCO band was detectable in the IR spectrum. 112 g of amino monomer of the formula (I) were obtained.

#### 15     Example 2

##### **Preparation of a gel bead polymer**

A solution of 5 g of polyvinyl alcohol (Mowiol® 40-88) and 1.5 g of disodium  
20     hydrogen phosphate in 130 ml of deionized water was introduced into a 250 ml reaction vessel with paddle stirrer, reflux condenser, thermometer, gas inlet tube and gas outlet tube. To this aqueous solution was added, at 20°C while stirring at 450 rpm, an organic solution of 7.91 g of N,N-dimethylaminoethyl methacrylate, 7 g of 2-hydroxyethyl methacrylate, 0.17g of triethylene glycol dimethacrylate, 0.225 g  
25     of 2,2'-azobis(2,4-dimethylvalereonitrile) and 37.5 g of chloroform over the course of 15 min. The mixture was gently flushed with nitrogen, and the temperature was raised to 67°C and kept at this temperature for 20 hours. After cooling, the bead polymer which had formed was separated off from the reaction liquor by decantation and freed of chloroform in vacuo at 50°C. 13.5 g of bead polymer with an average  
30     particle size of 20 µm and a swelling index of 5.1 measured at 25° C in water were obtained.

### Example 3

#### Preparation of a macroporous bead polymer

5

A solution of 42.5 g of polyvinyl alcohol (Moviol 40-88) and 12.75 g of disodium hydrogen phosphate in 1240 g of deionized water was introduced into a 2 liter reaction vessel with paddle stirrer, reflux condenser, thermometer, gas inlet tube and gas outlet tube. To this aqueous solution was added, at 20° C while stirring at 10 280 rpm (revolutions per minute), an organic solution of 23.71 g of N,N-dimethylaminoethyl methacrylate, 13.04 g of styrene, 10.67 g of divinylbenzene, 0.71g of 2,2'-azobis(2,4-dimethylvalereonitrile) and 31.62 g of hexane over the course of 30 min at 25°C. The mixture was gently flushed with nitrogen, and the temperature was raised to 66°C and kept at this temperature for 15 20 hours. The hexane was then distilled out at an internal temperature of 70 to 98° C. After cooling, the resulting bead polymer was separated from the reaction liquor by decantation and dried in vacuo at 50° C. 38 g of bead polymer with an average particle size of 15 µm and a specific surface area of 62.3 m<sup>2</sup>/g were obtained.

20

### Example 4

#### Preparation of a macroporous bead polymer

25

Example 3 was repeated employing an organic solution of 23.71 g of amino monomer from Example 1, 13.04 g of styrene, 10.67 g of divinylbenzene, 0.71g of 2,2'-azobis(2,4-dimethylvalereonitrile) and 38 g of hexane. 35 g of macroporous bead polymer with an average particle size of 25 µm and a specific surface area of 74 m<sup>2</sup>/g were obtained.

**Biological result**

- 100 µl of blood were mixed with 10<sup>5</sup> Caski cells. These cells contain human papillomavirus of type 16 (HPV).
- 5   • 10 µl of a 2.4% strength particle dispersion from Example 2 were added to this mixture..
- After addition of 200 µl of a suitable buffer, for example TE, incubation took place at room temperature for 5 min (TE represents 10 mmol of tris HCl and 1 mmol of ethylenediaminetetraacetic acid pH 7.4 in the final concentration).
- 10   • The particles were then sedimented in an Eppendorf centrifuge at 7 000 rpm for 3 min.
- Then 200 µl of a suitable lysis buffer, for example 0.5% IGEPAL CA-630® in TE (IGEPAL CA-630® is a nonionic detergent which can be purchased, for example, through Sigma, order number I 3021), were added to the sediment and incubated anew at room temperature for 5 min.
- 15   In place of IGEPAL CA-630® it is also possible, however, to employ other lysis buffers. Examples which may be mentioned are classical methods such as with protease K digestion and subsequent purification using phenol/chloroform or sodium lauryl sulfate solutions (Sigma order number: L 6026), for example as 0.5% strength aqueous solution.
- 20   • Renewed centrifugation and sedimentation took place in an Eppendorf centrifuge at 7000 rpm for 3 min.
- The particles were subsequently washed 2 x with a suitable buffer (for example TE). After the second washing step, the supernatant was discarded and further work was done only with the particles.
- 25   • The liberation of the nucleic acid bound to the particles took place by adjusting the pH to >12 by adding 1 µl of 0.5 normal NaOH.
- This was followed by incubation at room temperature for 15 minutes.
- After centrifugation (3 min, 7000 rpm in an Eppendorf centrifuge), the
- 30   concentration of the nucleic acid obtained was determined by a suitable

method, for example by analysis in a gel system, particularly preferably the "Submerged Gel Nucleic Acid Electrophoresis System", order No. 170 4406 from BIO-RAD (webpage: [www.bio-rad.com](http://www.bio-rad.com)).

Compared with beads for nucleic acid extraction known from the method of EP-A-0 707 077, distinctly better results were surprisingly achieved with the method of the invention and the bead polymers used therein.

- 15 µl of the supernatant obtained in this way were then employed in an HPV-specific PCR (polymerase chain reaction), that is to say primer was employed in a PCR which are specific for human papillomaviruses (HPV). On analysis of the result in a gel system it was possible to identify the expected nucleic acid band clearly and distinctly.



**Patent claims**

1. A method for isolating nucleic acids from a sample comprising the following steps
- 5
- A) mixing the sample with a water-insoluble polymer which is not ionic in the basic and neutral range, at a pH of 7 or less, with the nucleic acids being adsorbed,
- 10
- B) separating off the water-insoluble polymer and
- C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, with the adsorbed nucleic acids being liberated,
- 15
- characterized in that the water-insoluble polymer is a bead polymer with an average particle size of from 3 to 100  $\mu\text{m}$  and consists of polymerized units of
- a) 5 to 98% by weight of amino monomer
- b) 0.3 to 30% by weight of crosslinker and
- 20
- c) 0 to 93% by weight of vinyl monomer.
2. A method as claimed in claim 1, characterized in that the biological material is lysed after method step A).
- 25
3. A method for isolating nucleic acids comprising steps A), B) and C) as claimed in claims 1 and 2, characterized in that the polymer is a water-soluble, macroporous bead polymer with an average particle size of from 3 to 100  $\mu\text{m}$  and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$  and consists of polymerized units of
- 30
- a) 5 to 98% by weight of amino monomer

- 20 -

- b) 0.3 to 30% by weight of crosslinker and
- c1) 0 to 93% by weight of hydrophobic vinyl monomer

or in that the water-insoluble polymer consists of

bead polymer which is able to swell in water well and has an average particle size of from 3 to 100  $\mu\text{m}$ , and which consists of polymerized units of

- a) 5 to 79.5% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer.

4. A water-insoluble, macroporous bead polymer with an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$ , characterized in that it consists of polymerized units of

- a) 5 to 98% by weight of amino monomer
- b) 2 to 30% by weight of crosslinker and
- c1) 0 to 93% by weight of hydrophobic vinyl monomer.

5. A bead polymer which is insoluble in water but swellable in water and has an average particle size of from 3 to 100  $\mu\text{m}$ , characterized in that it consists of polymerized units of

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer.

6. A method for preparing water-insoluble, macroporous bead polymers with an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to

1000 nm and a specific surface area measured by the BET method of from 5 to 500 m<sup>2</sup>/g, characterized in that a mixture of

- a) 5 to 98 parts by weight of amino monomer
- b) 2 to 30 parts by weight of crosslinker
- c1) 0 to 93 parts by weight of hydrophobic vinyl monomer
- d) 10 - 150 parts by weight of porogen and
- e) 0.1 - 2.5 parts by weight of free-radical former

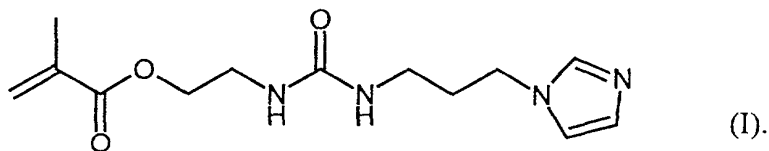
is dispersed in an aqueous medium using a protective colloid, subsequently the resulting dispersion is polymerized by heating to the decomposition temperature of the free-radical former and, after the polymerization has taken place, the porogen is removed by extraction and/or evaporation.

7. A method for preparing bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100  $\mu$ m, characterized in that a mixture of

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer
- d) 10-150 parts by weight of solvent and
- e) 0.1 - 2.5 parts by weight of free-radical former

is dispersed in an aqueous medium using a protective colloid, subsequently the resulting dispersion is polymerized by heating to the decomposition temperature of the free-radical former and, after polymerization has taken place, the solvent is removed by extraction and/or evaporation.

8. An amino monomer of formula (I)



9. A method for preparing amino monomer of the formula (I) as claimed in claim 8, characterized in that 2-isocyanatoethyl methacrylate is reacted with 3-aminopropylimidazole.

10. The use of water-insoluble, macroporous bead polymers with an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm and a specific surface area measured by the BET method of from 5 to 500  $\text{m}^2/\text{g}$ , consisting of polymerized units of

- a) 5 to 98% by weight of amino monomer
- b) 2 to 30% by weight of crosslinker
- c1) 0 to 93% by weight of hydrophobic vinyl monomer

or of bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100  $\mu\text{m}$ , consisting of polymerized units of

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer,

for isolating nucleic acids from a sample.

11. A composition for isolating nucleic acids from a sample comprising water-insoluble macroporous bead polymers with an average particle size of from 3 to 100  $\mu\text{m}$ , a pore diameter of from 10 to 1000 nm and a specific surface area

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measured by the BET method of from 5 to 500 m<sup>2</sup>/g, consisting of polymerized units of

- a) 5 to 98% by weight of amino monomer
- b) 2 to 30% by weight of crosslinker
- c1) 0 to 93% by weight of hydrophobic vinyl monomer

or of bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100 µm, consisting of polymerized units of

- a) 5 to 79.7% by weight of amino monomer
- b) 0.3 to 10% by weight of crosslinker and
- c2) 10 to 93% by weight of hydrophilic vinyl monomer.

**COMBINED DECLARATION AND POWER OF ATTORNEY**

ATTORNEY DOCKET NO

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name. I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

**METHOD FOR ISOLATING NUCLEIC ACIDS**

the specification of which is attached hereto,

or was filed on **February 9, 2000**

as a PCT Application Serial No. **PCT/EP00/01028**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s), the priority(ies) of which is/are to be claimed:

**199 07 023.7**  
(Number)

**Germany**  
(Country)

**February 19, 1999**  
(Month/Day/Year Filed)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose the material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

(Application Serial No.)

(Filing Date)

(Status)

(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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